Pre- and Postharvest Calcium Treatment of Apple Fruit and its Effect on Quality

W.S. Conway[1], C.E. Sams[2], K.D. Hickey[3]
[1]Product Quality and Safety Laboratory, Henry A.Wallace Beltsville Agricultural Research Center, United States Department of Agriculture, Beltsville, USA
[2]Department of Plant and Soil Sciences, The Universioty of Tennessee, Knoxville, USA
[3]Department of Plant Pathology, The Pennsylvania State University, Fruit Research and Extension Center. Biglerville, USA

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Abstract

Calcium is thought to be the most important mineral element determining fruit quality. It seems to be especially important in apples where it has been shown to reduce metabolic disorders. Calcium in adequate amounts helps to maintain apple fruit firmness and decreases the incidence of physiological disorders such as water core, bitter pit and internal breakdown. Postharvest decay may also be reduced by increasing the calcium content of apple fruit. The major problem is getting enough calcium into the fruit to have the desired effects. Soil treatments with calcium to increase fruit calcium concentration have often met with very little success. The direct application of calcium to the fruit is the most effective method for increasing fruit calcium content. This can be accomplished by preharvest sprays or postharvest dips or vacuum or pressure infiltration. Although calcium sprayed on apples on the tree can increase calcium content of the fruit, direct postharvest treatment of the fruit in solutions of calcium chloride can be even more effective. Pressure infiltration with solutions of calcium chloride can increase the calcium concentrations of apple fruit more effectively than vacuum infiltration which is more effective than dipping. Both pre- and postharvest calcium treatment methods have inherent problems. Inadequate calcium uptake is a problem in some cases in that not enough calcium is taken into the fruit to have a positive effect. In other cases, excessive calcium is taken into the fruit and injury results. Developing a commercially acceptable method of successfully increasing calcium concentration in fruit is a continuing challenge. In the meantime, orchard management to optimize fruit calcium uptake in the preharvest environment combined with supplemental postharvest application is the best strategy to prevent losses due to low fruit calcium.

INTRODUCTION

Calcium (Ca) plays an extremely important role in plant growth and development and in maintaining and modulating various cell functions (Hanson, 1984; Palta, 1996). It is necessary to maintain membrane stability and is an integral part of the cell wall where it provides rigidity.

It also has importance as an intracellular secondary messenger (Poovaiah and Reddy, 1993). Enhancing the calcium content of apple fruits can be very beneficial in maintaining fruit quality during storage. Calcium treatments can maintain fruit quality by reducing softening (Mason, et al., 1975), internal breakdown and respiration (Bangerth et al., 1972), bitter pit (Reid and Padfield, 1975), ethylene production (Sams and Conway, 1984), and decay (Conway and Sams, 1983). Postharvest losses due to *Gloeosporium* spp. were reduced in fruit sprayed preharvest with Ca sprays compared to unsprayed apples (Sharples and Johnson, 1977). Postharvest infiltration of $CaCl_2$ solutions into apple fruit significantly reduced decay caused by *Penicillium expansum* Link, *Botrytis cinerea* Pers.:Fr., and *Glomerella cingulata* (Stoneman) Spauld. & Schrenk (Conway and Sams, 1983; Conway et al., 1991; Conway et al., 1992). The major problem is raising the Ca concentration of the fruit to a sufficient level to have the desired results. It has been

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postulated that calcium tissue concentrations should exceed 250 µg g^{-1} dry weight to control many calcium-related physiological disorders such as breakdown and bitter pit (Meheriuk and Moyls, 1989). In order to affect firmness or decay significantly, however, it is necessary to raise the level of tissue calcium to 800-1000 µg g^{-1} dry weight (Sams and Conway, 1984; Conway and Sams, 1985). Concentrations significantly higher than 1000 µg g^{-1} may cause surface injury to the fruit. Fertilization regimes, preharvest sprays, and postharvest treatment have all been tried in an effort to increase fruit tissue calcium in order to have a positive effect on fruit quality in storage. This paper will discuss some of the more recent literature on increasing the Ca concentration of apple fruit and the relationship this increased Ca has to physiological and particularly pathological maladies.

FRUIT TREE SPRAYS

Earlier work on tree sprays with $Ca(NO_3)_2$ indicated that these sprays reduced both internal breakdown and bitter pit as well as decay caused by *Gloeosporium perennens* Zeller et Childs (Sharples and Johnson, 1977). In the last decade, researchers have continued efforts to increase Ca concentration of fruit by spraying with various calcium compounds. Fruit sprays on pears have improved fruit quality especially in critical years when fruit Ca concentrations are low due to record high seasonal temperatures or freeze injury to fruit trees in winters prior to Ca sprays. Ca concentrations were increased substantially in with $CaCl_2$ sprays from early June to August (Raese et al., 1999). This Ca increase resulted in firmer fruit, a lower incidence of cork spot and brown core, and lower ethylene production and respiration. As a result, fruit quality was improved and shelf life enhanced. An extensive study was conducted over several years in Finland on preharvest Ca sprays of eight apple cultivars (Dris and Niskanen, 1999). In this study, $CaCl_2$ sprays significantly increased fruit Ca content and firmness in only one of the cultivars and treatment effects in general varied among cultivars and growing seasons. In our most recent study to determine Ca uptake from preharvest fruit sprays in Pennsylvania, 'Golden Delicious', 'Nittany', and 'Red Delicious' were treated with 8 postbloom applications of $CaCl_2$ and were analyzed for tissue Ca content after 3 months in storage at 0 °C. The fruit tissue analyzed for Ca concentration was in the layer 2-4 mm beneath the peel. The Ca concentration in 'Golden Delicious' fruit was increased by 100% (from 169 $\mu g \cdot g^{-1}$ to 340 $\mu g \cdot g^{-1}$), in 'Nittany' by 55% (from 156 $\mu g \cdot g^{-1}$ to 241 $\mu g \cdot g^{-1}$) and in 'Red Delicious' by 92% (from 175 $\mu g \cdot g^{-1}$ to 334 $\mu g \cdot g^{-1}$). However, there was not a sufficient increase in Ca concentration to significantly maintain fruit firmness in any of the cultivars during storage. Fruit were also wound inoculated at the time of removal from storage with *Botryosphaeri obtusa* (Schwein.) Shoemaker, *G. Cingulata*, and P. *expansum*. The effects of these Ca sprays in reducing the level and severity of the fruit rot infections were sporadic and generally not statistically significant. In a similar experiment, there were no significant treatment effects on reducing decay following wound inoculations with the three pathogens (Hickey et al., 1995). However, in field trials where apples received three weekly dilute applications of Ca solutions, fruit treated with Ca salts and inoculated with *Colletotrichum gloeosporioides* Penz. or *Colletotrichum acutatum* J. H. Simmonds exhibited lower incidences of infection when compared to control fruit (Biggs, 1999). These experiments demonstrated that calcium salts have suppressive activity against the bitter rot pathogens and can be used as part of a disease management program. Suppressive effects included reduced fungal germ tube growth, reduced mycelial growth in vitro, and reduced severity of infection of host tissue pretreated with calcium. Incubating *B. cinerea* spores in increasing concentrations of $CaCl_2$ (4-26 g L^{-1}) resulted in decreased spore germination and germ tube length. This inhibitory effect could be overcome by the addition of glucose to the germination medium (Wisniewski et al., 1995). Another in vitro study was conducted to determine the direct effect of calcium on the mycelial cell walls of *B. cinerea* (Chardonnet et al., 1999). *B. cinerea* was grown on Richard's solution containing different amounts of $CaCl_2$. The resulting thickening of the fungal cell wall, which occurred when Ca concentrations increased, could retard the elongation phase of the hyphae and result in a loss of cell wall

elasticity. This thickening of the fungal cell wall caused by the Ca ion may be an important factor in the host-pathogen relationship. The direct effect of increased Ca on the fungus, however, may be considered a secondary effect. The primary effect seems to result from the accumulation of Ca in the cell wall. The resulting Ca bridges in the cell wall between pectic acids or between pectic acids and other polysaccharides hinder accessibility to the cell wall by pectolytic enzymes, such a polygalacturonase, produced by fungal pathogens during decay (Conway et al., 1988). This relationship between Ca and the cell wall also at least partially explains the maintenance of fruit firmness by increased tissue Ca.

POSTHARVEST TREATMENTS

Postharvest dipping, vacuum infiltration, and pressure infiltration of Ca solutions have been used with varying degrees of success to maintain fruit quality (Sams and Conway, 1984; Scott and Wills, 1979; Scott et al., 1985). Vacuum or pressure infiltration of $CaCl_2$ solutions was superior to dips for control of bitter pit in Australia and New Zealand (Scott and Wills, 1979). Experimentally, pressure infiltration with solutions of $CaCl_2$ for 2 minutes at 69 kPa can increase the Ca concentration of fruit more effectively than vacuum infiltration at 33 kPa or dipping for 2 minutes (Conway and Sams, 1983). Most research on pressure-infiltration of high concentrations of Ca in apple fruit has been conducted on small lots of fruit in a laboratory environment, where sanitation and fruit treatment were strictly controlled. In commercial situations, where larger amounts of fruit are to be treated, conditions are less optimal. Therefore, a pilot test was conducted over a three year period in Pennsylvania to determine the feasibility of infiltration of apples at harvest with $CaCl_2$ on a commercial basis (Conway, et al., 1994). Each year, fruit from three different orchards were delivered to a commercial facility where the fruit were pressure infiltrated (103 kPa, 6 minutes) with various concentrations of $CaCl_2$ solutions. In the first year, 'Golden Delicious' fruit were infiltrated with 0, 2, or 4% $CaCl_2$ solutions. In the second two years, 'Red Delicious' apples were infiltrated with 0, 4, 6, or 8% $CaCl_2$ solutions. All tests included a nontreated control. The fruit Ca concentration, analyzed from tissue taken 2-4 mm directly beneath the peel, was quite variable each year depending upon the orchard from which the fruit were taken. In general, the yearly uptake pattern was similar in that fruit from one orchard took up a large amount of Ca, the fruit from a second orchard took up a moderate amount of Ca, and the fruit from a third orchard took up a low amount of Ca. In summary, the 'Golden Delicious' fruit infiltrated with 0, 2, or 4% $CaCl_2$ solutions had flesh Ca concentrations of 175, 1223, and 1536 $\mu g \cdot g^{-1}$ dry weight, respectively, with the resulting fruit firmness after 6 months storage at 0 °C being 60.8, 70.7 and 73.3 N, respectively. 'Red Delicious' fruit infiltrated with 0, 4, 6, or 8% $CaCl_2$ solutions had flesh Ca concentrations of 156, 902, 909, and 1260 $\mu g \cdot g^{-1}$ dry weight, respectively, with a fruit firmness of 67, 71, 75, and 75 N, respectively. The fruit for both the spray study, discussed in the previous section, and the pressure infiltration pilot test were taken from the same fruit growing area in southern Pennsylvania. Yet, the highest Ca flesh concentration resulting from the Ca sprays was approximately 340 $\mu g \cdot g^{-1}$ dry weight in both 'Golden Delicious' and 'Red Delicious' fruit, while the highest Ca concentration resulting from pressure infiltration was 1536 $\mu g \cdot g^{-1}$ dry weight in 'Golden Delicious' apples and 1260 $\mu g \cdot g^{-1}$ dry weight in the 'Red Delicious' fruit. As previously stated, the general guideline for Ca flesh concentration is approximately 250 $\mu g \cdot g^{-1}$ dry weight to control many Ca related physiological maladies such as bitter pit and breakdown, and 800 $\mu g \cdot g^{-1}$ dry weight to significantly affect firmness and decay. The spray program increased Ca concentration sufficiently to alleviate potential physiological disorders, but not enough to affect firmness or decay. The Ca concentrations resulting from pressure infiltration exceeded the required amounts for maintaining fruit firmness and reducing decay.

However, several problems are inherent in using this procedure commercially. Different cultivars take up different amounts of $CaCl_2$, as do fruit of the same cultivar of different maturities or from different orchards and growing seasons. Another potential

problem is the possibility of an increase in infection and fruit surface injury. In our study, 'Golden Delicious' was very susceptible to skin injury while 'Red Delicious' was much less so. However, 'Golden Delicious' fruits that were pressure infiltrated with $CaCl_2$ solutions and then waxed to reduce water loss during storage showed no peel injury (Saftner et al., 1998). The majority of the Ca injury was to the peel area. Such an injury would prohibit sale as fresh fruit, but the fruit would still be suitable for processing.

Sensory evaluation studies were conducted on 'Golden Delicious' apples pressure infiltrated with 2% $CaCl_2$ solutions after fruit were stored for 6 months (Klein, et al., 1998). Ca-treated fruit were perceived as crisper, sweeter, and overall more acceptable than untreated fruit. The conclusion of this one study was that in addition to conferring physiological benefits on stored apples, Ca treatments did not decrease and may even improve consumer acceptability.

THE ROLE OF CALCIUM IN INTEGRATED PEST MANAGEMENT

Attempts to find alternatives to chemical control to reduce losses from postharvest decays have been ongoing for some time. Many fungi are becoming more resistant to commonly used fungicides and there is an increasing demand by consumers to reduce chemical residues on produce due to health and environmental concerns. Alternatives to chemical control, when used alone, are generally less effective than fungicides. For this reason, it may be necessary to combine several of these alternatives to equal the effectiveness of fungicides. Methods should be chosen which complement each other in order to minimize the shortcomings of each. An integrated program using Ca sprays was developed to reduce decay of 'Bosc' pears (Sugar et al., 1994). A combination of preharvest Ca sprays followed by postharvest treatments of various combinations of a biocontrol agent, controlled atmosphere, and one tenth the label rate of the fungicide thiabendazole (Mercect 340F) significantly reduced decay caused by *P. expansum*, and completely controlled that caused by *Phialophora malorum* (M. N. Kidd & A. Beaumont) McColloch. Postharvest use of Ca and other alternatives also shows promise. Combining the antagonist *Pseudomonas syringae* van Hall with Ca infiltration reduced the incidence of decay and lesion size caused by *P. expansum* more effectively than either treatment alone after 3 and 6 months in storage (Janisiewicz, et al., 1998). In a related postharvest study to reduce decay caused by *P. expansum*, apples were heat treated (38 °C, 4 days) followed by Ca infiltration and then treatment with the antagonist *P. syringae* (Conway et al., 1999). Once again, the treatment combination was more effective than any of the treatments alone. Heat treatment is phytosanitary in that it significantly reduces the pathogen population on the fruit surface but it provides little residual protection. The residual protection provided by Ca and the antagonist adds to the control provided by the heat treatment. The strategy of heat treating fruit, followed by Ca infiltration and then treatment with an antagonist may be a useful alternative to fungicides for postharvest decay control.

CALCIUM FORMULATIONS AND COMPARISONS WITH OTHER CATIONS

Ca in various formulations has been applied to fruit in an attempt to increase tissue Ca concentration. Of eight Ca compounds tested, only $CaCl_2$ and Ca lactate were effective in reducing bitter pit, with Ca lactate being approximately 50% as effective as the chloride formulation (Scott and Wills, 1979). When comparing postharvest pressure infiltration of $CaCl_2$ with that of Stopit, a proprietary $CaCl_2$ solution, both resulted in significant increases in tissue Ca concentration in an amount directly correlated with the concentration of Ca in the treatment solution (Beavers et al., 1994). Thus, the decision to use either $CaCl_2$ or Stopit should be based on economics, due to the higher cost of Stopit, because the two compounds act essentially the same. In this same study, treatment with Ca chelate resulted in no increase in Ca tissue content but did cause extensive injury to the fruit. Fruit sprays with Ca chelate from various sources were found to be as effective as $CaCl_2$ for improving fruit Ca concentrations, but these materials have much lower Ca concentrations than $CaCl_2$ (Bramlage, 1987). Therefore, larger quantities must be used to

achieve equivalent results and the costs are more and greater than those for $CaCl_2$. Various cations other than Ca, especially divalent cations, have been implicated in alleviating physiological and pathological problems of stored produce, thereby maintaining quality. In a study to determine the effects of postharvest infiltration of chloride formulations of Ca, magnesium, or strontium on various postharvest maladies, Ca was found to be the optimum cation for alleviating postharvest physiological and pathological problems (Conway and Sams, 1987). Also, those fruit infiltrated with $MgCl_2$ solutions exhibited injury, the symptoms of which resembled bitter pit. In bean tissue infected with *Rhizoctonia solani* Kuhn, tissue maceration by polygalacturonase was greatly reduced in the presence of barium and Ca; it was less inhibited by magnesium; and was not significantly influenced by the monovalent cations potassium or sodium (Bateman, 1964). In other studies, Ca was more important than magnesium, potassium, nitrogen, and phosphorus in influencing apple quality (Faust and Shear, 1968; Bramlage et al., 1985). In comparing the effects of $CaCl_2$ with those of $MgCl_2$ on spore germination and germ tube growth of the apple pathogen *B. cinerea*, $CaCl_2$ decreased both parameters while $MgCl_2$ had no effect on either (Wisniewski et al., 1995). The research cited here not only indicates the superiority of Ca in alleviating various maladies over other cations, but also that it is the Ca cation rather than the chlorine anion which is responsible.

UPTAKE OF CALCIUM INTO APPLE FRUIT

During treatment of apples with $CaCl_2$ solutions, Ca probably enters the fruit primarily through the lenticels (Betts and Bramlage, 1977), but cracks in the cuticle and epidermis may also be important pathways (Glenn and Poovaiah, 1985). These cracks are especially prevalent in 'Golden Delicious' fruit early in the growing season, and increase in width and number as the fruit enlarge and mature (Meyer, 1944). At maturity, the cracks on the surface of the fruit are larger and form a network (Faust and Shear, 1972). Studies on the cuticle permeability to $CaCl_2$ solutions indicated that both permeability and the number of cracks in the cuticle increased as fruit developed (Glenn and Poovaiah, 1985). Late preharvest Ca sprays increased apple fruit Ca content more than early sprays (Stahy, 1986). In a more recent study, apples were nontreated or pressure infiltrated with distilled water or with $CaCl_2$ solutions 2 weeks before prime harvest, at optimum harvest, or after 2 , 4 , or 6 months in storage at 0 °C (Roy et al., 1999). Examination of the fruit surface with low -temperature scanning electron microscopy revealed that cracks in the epicuticular wax became wider and deeper as storage duration increased. After 6 months of storage, the cracks extended through the cuticle. The development of cracks and other surface irregularities during the latter part of the growing season may play a significant role in Ca penetration into apple fruit. A study was conducted of various nonionic surfactants on pressure infiltration of Ca into apples (Roy et al., 1996). The physio-chemical activity of these surface active agents on the fine structure of the epicuticular wax was also examined. Removing lipid-soluble portions of the epicuticular wax layer on the apple surface resulted in significantly increased uptake of Ca during infiltration. Using surfactants as surface agents to solubilize components of the epicuticular wax structure would be a way of enhancing Ca uptake.

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